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Review

Spectral Organization of Ommatidia in Flower-visiting Insects[†]

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ABSTRACT

This article reviews recent advances of studies on the spectral organization of the compound eye in lepidopteran and hymenopteran insects. The compound eyes consist of ommatidia, which contain a set of photoreceptor cells. The common feature is that the ommatidia can be divided into three types, according to the combination of spectral classes of photoreceptors. Honeybees and nymphalid butterflies provide the simplest case with three photoreceptor classes having peak sensitivities in the ultraviolet (UV), blue (B), or green (G) wavelength region. These receptors populate the ommatidia in fixed combinations. In type I ommatidia, the main eight photoreceptors consist of one UV, one B, and six G receptors. Type II has two UV and six G receptors, and type III has two B and six G receptors. The organization is basically retained in all insect species studied so far, although some butterflies have more than six classes of spectral receptors, including those sensitive in the violet and red wavelength regions. To acquire these additional receptors, two distinct strategies are applied: the multiple opsin strategy, taken by the Japanese yellow swallowtail butterfly *Papilio xuthus*, and the filter strategy, used by the small white butterfly *Pieris rapae*.

INTRODUCTION

Compound eyes consist of numerous, anatomically identical units called ommatidia. In most flower-visiting insects, an ommatidium contains nine photoreceptor cells with various spectral sensitivities. Not all ommatidia are furnished with identical sets of spectral receptor classes, however, even in a single compound eye: the ommatidia are spectrally heterogeneous. Ommatidial heterogeneity has been repeatedly reported for many years in several insect species (1–4), but only recently, the details have been clarified for a number of butterflies (5–11). Here, we survey recent advances in the study of ommatidial heterogeneity in insects, and we compare the heterogeneity from an evolutionary point of view.

BASIC DESIGN

The cell membrane of insect photoreceptors is locally invaginated into numerous microvilli, forming the rhabdomere. The

rhabdomeres of the photoreceptor cells of one and the same ommatidium together form the rhabdom. The rhabdom of diurnal insects such as honeybees and butterflies is a slender, cylindrical structure that acts as an optical waveguide (Fig. 1). The microvillar membranes are packed with visual pigment molecules that absorb light propagating in the rhabdom waveguide. A visual pigment molecule consists of an opsin protein with an 11-*cis* retinal attached to it as the chromophore. The amino acid sequence of the opsin tunes the absorption spectrum of the visual pigment, and thus the visual pigment molecule primarily determines the spectral sensitivity of the photoreceptor. Sets of photoreceptors with different spectral sensitivities provide the physiological basis of color vision.

As Karl von Frisch first demonstrated the visual capacities of the honeybee, *Apis mellifera*, this species has been the classic model system for studying mechanisms of color vision, and indeed the first identification of different spectral receptors in a compound eye was carried out in this insect species (12). Intracellular electrophysiology was later applied to the compound eye of *Apis* to unravel the spectral sensitivities of the nine photoreceptor cells (R1–9) that compose an ommatidium. At least three classes of spectral receptors were identified, with peak sensitivities in the ultraviolet (UV), blue (B), and green (G) wavelength region (13). The localization of these spectral receptors has been studied by electrophysiology coupled with dye injection (14) as well as by analyzing light-induced ultrastructural changes in specific photoreceptors (15). The early studies thus led to the commonly accepted view that all ommatidia of *Apis* contain the same set of spectral receptors, consisting of three UV, two B, and four G receptors (16).

The development of powerful molecular biological methods has significantly stimulated the study of insect color vision, and has yielded a rapidly growing data set of primary structures of visual pigment opsins (Fig. 2). Three visual pigments, corresponding to the UV, B, and G receptors, were identified in *A. mellifera*: AmUV, AmB, and AmG (17–19). It thus became possible to “map” the spectral receptors in the entire retina by *in situ* hybridization, where opsin mRNAs are labeled in histological sections. We recently applied this method to the retina of *Apis*, and found that there are three distinct types of ommatidia, refuting the common assumption that the ommatidia contain identical sets of spectral receptors (20). As an example, Fig. 3 shows a region of the bee compound eye in three consecutive sections. The sections were

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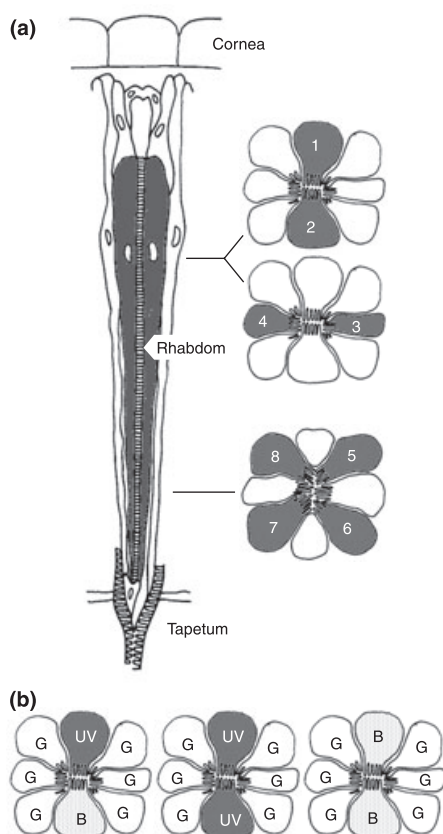


Figure 1. Schematic diagram of an ommatidium of nymphalid butterflies. (a) Longitudinal and transverse view through the distal and the proximal region. Of nine photoreceptor cells, eight (1–8) contribute photoreceptive microvilli to the rhabdom along the entire length of the rhabdom. The ninth photoreceptor adds a few microvilli at the base of the rhabdom. The tapetum is a tracheole folded into a stack of layers of air and cytoplasm, and thus functions as an interference mirror. (b) The spectral sensitivities of photoreceptors 1–8, based on the results of *in situ* hybridization of opsin mRNAs, are indicated: UV, ultraviolet receptor; B, blue receptor; G, green receptor.

labeled with cRNA probes that specifically hybridize to the AmUV (Fig. 3a), AmB (Fig. 3b), or AmG (Fig. 3c) mRNAs, respectively. The labeling pattern demonstrates that there are three ommatidial types. The ommatidia of so-called type I contain one UV and one B receptor, type II ommatidia contain two UV receptors, and type III ommatidia contain two B receptors. Similar patterns with the same three ommatidial types have been concluded to exist in the retina of the bumble bee *Bombus impatiens* (21).

The molecular organization of the compound eye and its photoreceptors has been documented also in some butterflies and moths. Specifically, well-studied species are the painted lady (*Vanessa cardui*, Nymphalidae) (11), the tobacco hawk-moth (*Manduca sexta*, Sphingidae) (22), the small white (*Pieris rapae*, Pieridae) (23,24), and the Japanese yellow swallowtail (*Papilio xuthus*, Papilionidae) (6). The tiger swallowtail, *Papilio glaucus* (25,26), and the monarch, *Danaus plexippus* (10), also provide useful information, but the data are still somewhat fragmentary. *Vanessa* and *Manduca* appear to have ommatidia with the same photoreceptor composition as those of *Apis*. They have three visual pigment opsins, corresponding to UV, B, and G receptors. Localization of the opsin mRNAs

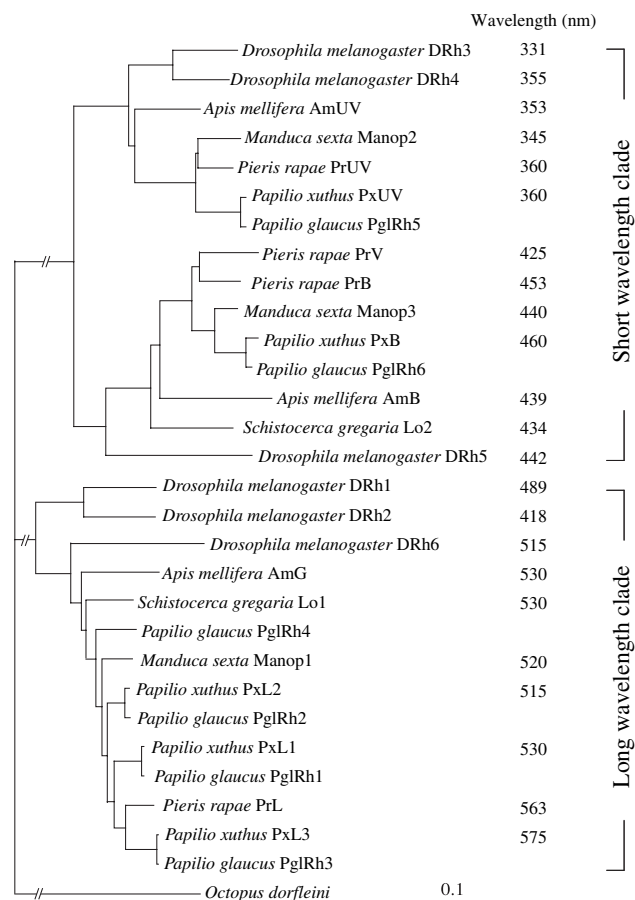


Figure 2. Phylogenetic relationship of insect visual pigment opsins. Data accession numbers: *Apis mellifera* (AmB = AF004168; AmG = BK005514; AmUV = AF004169), *Drosophila melanogaster* (Rh1 = K02315; Rh2 = M12896; Rh3 = M17718; Rh4 = P08255; Rh5 = U67905; Rh6 = Y00043), *Manduca sexta* (Manop1 = L78080; Manop2 = L78081; Manop3 = AD001674), *Papilio glaucus* (PgIRh1 = AF077189; PgIRh2 = AF077190; PgIRh3 = AF067080; PgIRh4 = AF077193; PgIRh5 = AF077191; PgIRh6 = AF077192), *Papilio xuthus* (PxB = AB028217; PxUV = AB028218; PxL1 = AB007423; PxL2 = AB007424; PxL3 = AB007425), *Pieris rapae* (PrL = AB177984; PrUV = AB208673; PrV = AB208675; PrB = AB208674), *Schistocerca gregaria* (Lo1 = X80071; Lo2 = X80072). The peak wavelengths were taken from Salcedo *et al.* (48) (*Drosophila*), Townson *et al.* (18) (*Apis*), White *et al.* (22) (*Manduca*), Engels *et al.* (49) (*Schistocerca*) and Arikawa (6) (*Papilio*).

by *in situ* hybridization revealed three types of ommatidia, according to the pattern of opsin expression corresponding to short wavelengths-absorbing visual pigments in two of the eight main photoreceptor cells: UV/B (UV and B opsin mRNAs) in type I, B/B in type II, and UV/UV in type III. All six other photoreceptors in all three ommatidial types express the G opsin mRNAs (Fig. 1b).

The retinal organization of *Vanessa* and *Manduca* is straightforward, because there is a one-to-one relationship between the visual pigments and the photoreceptor spectral sensitivities. However, the situation is not always so simple: *Pieris rapae* has four opsins and no less than seven classes of spectral receptors, and *P. xuthus* has five opsins and six classes of receptors. The cellular organization of the ommatidium is also somewhat different. In *Vanessa* and other nymphalids, the eight main photoreceptors contribute their microvilli to the

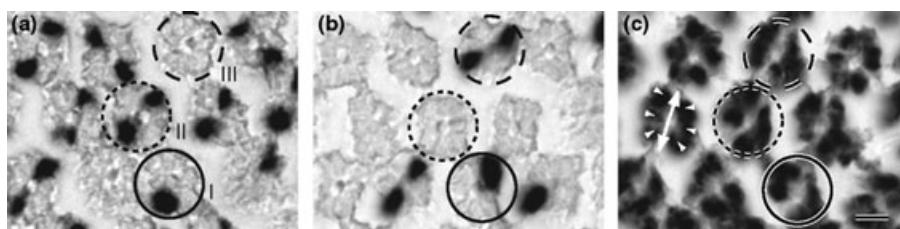


Figure 3. *In situ* hybridization of mRNA encoding AmUV (a), AmB (b) and AmG (c) opsins in three consecutive sections of the honeybee *Apis mellifera*. Three types of ommatidia, I (solid circles), II (dotted circles) and III (broken circles), are evident. The ommatidial axis (white arrow in c) is variable because of the ommatidial twist. Scale: 5 µm.

rhabdom over its entire length (Fig. 1) (27,28). In *Pieris* and *Papilio*, the rhabdom is tiered, however. The microvilli of four distal photoreceptors, R1-4, make up the distal half of the rhabdom, and four proximal photoreceptors, R5-8, form the proximal half. The so-called basal receptor, R9, adds a small number of microvilli at the very base of the rhabdom. A special feature of the butterfly ommatidium is the tapetum, created by tracheoles proximally of the rhabdom, which exists in all butterfly families except the papilionids (Fig. 1a). The tapetum reflects light that has propagated throughout the whole length of the rhabdom, without having been absorbed back into the rhabdom, so that it has another chance to be absorbed.

PIERIS: PHOTORECEPTOR DIVERSIFICATION THROUGH THE ACTION OF SCREENING FILTER PIGMENTS

Extensive studies of the anatomy, the molecular biology of the visual pigment, and the spectral sensitivities of the photoreceptors via intracellular electrophysiology have yielded a detailed insight into the retinal properties of the small white butterfly, *Pieris rapae*. The ommatidia of *Pieris* have a clear morphological heterogeneity according to a characteristic pigmentation of the proximal photoreceptors, R5-8, in the

main, dorso-ventral part of the eye (7). Transverse light-microscopical sections reveal pigment clusters as four colored spots around the rhabdom (Fig. 4b). The four pigment spots are arranged in a trapezoidal (type I), square (type II) or rectangular (type III) fashion. The color of the pigment is pale-red in type I and type III ommatidia, whereas it is deep-red in type II. In males, the type II ommatidia are special, because they contain in the distal portion of the rhabdom some unidentified substance that fluoresces under violet epi-illumination (Fig. 4c) (24).

By combining electrophysiology with histology, we determined the spectral sensitivities of the different photoreceptor classes in all three types of ommatidia of *Pieris rapae* (7–9). The results are summarized in Fig. 4. A striking feature is the extremely aberrant spectral sensitivities of the long wavelength receptors. There are two distinct receptor classes with peak sensitivities above 600 nm; a red (R) receptor peaking at 620 nm and a deep-red (dR) receptor peaking at 640 nm (Fig. 5a). The peak wavelength of the dR receptor, 640 nm, is the longest among insect photoreceptors identified so far. The R and dR receptors presumably endow *Pieris* with a pronounced ability to discriminate subtly differing red colors.

Quite surprisingly, it turned out that the G, R, and dR receptors all express one and the same visual pigment, PrL (*Pieris rapae* Long wavelength type, Fig. 5b,c). The spectral

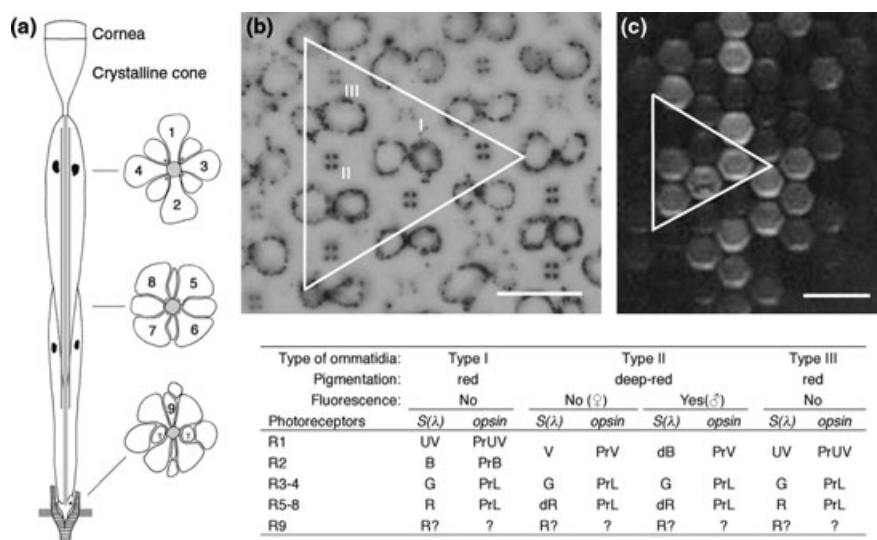


Figure 4. Anatomy of the *Pieris* eye. (a) The ommatidia of *Pieris* are tiered with four distal (1–4), four proximal (5–8), and one basal (9) photoreceptor. (b) Transverse section through the middle layer, showing three types of ommatidia distinguishable by the perirhabdomal pigment clusters. (c) Fluorescence picture of male ommatidia under 420 nm epi-illumination. Same eye as in (b). The white triangle indicates the same set of ommatidia as the set enclosed by the white triangle in (b), thus demonstrating that type II ommatidia fluoresce. Table: Spectral sensitivities and visual pigments of photoreceptors R1-9 in the three types of the ommatidia. UV, ultraviolet; V, violet; B, blue; dB, double-peaked blue; G, green; R, red; dR, deep-red. For visual pigments, see Fig. 2. Scales: 10 µm (b) and 50 µm (c).

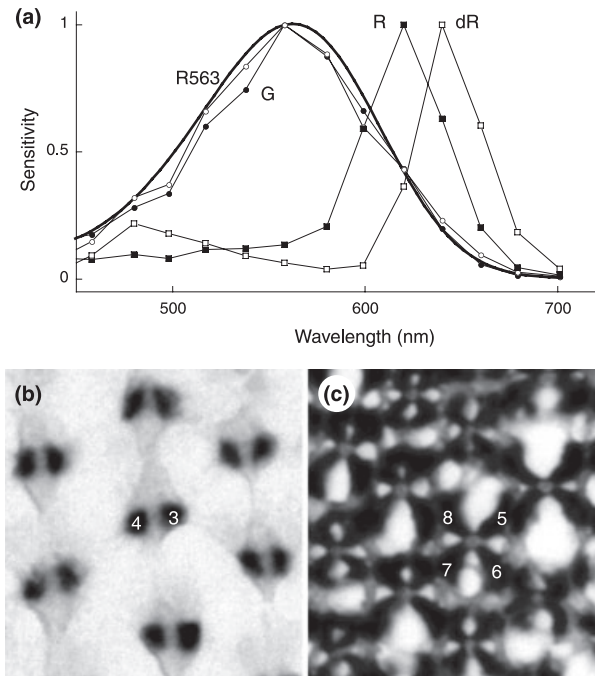


Figure 5. Long-wavelength photoreceptors of *Pieris*. *In situ* hybridization of the PrL mRNA. (a) Spectral sensitivities of G (open and filled circles, R3-4), R (filled squares, R5-8 of type I and III), and dR (open squares, R5-8 of type II) receptors. Solid thick line is the predicted absorption spectrum of a visual pigment peaking at 563 nm. (b) Distal tier, photoreceptors R3 and R4 are labeled in all ommatidia. (c) Proximal tier, photoreceptors R5-8 are labeled in all ommatidia. Numbers indicate photoreceptor position. See also Fig. 4a.

sensitivities of the green-sensitive receptors R3 and R4 well match the absorption spectrum expected for a visual pigment absorbing maximally at 563 nm (therefore called rhodopsin R563), indicating that PrL is a R563 (29,30). This finding suggested that the red or deep-red pigmentation around the rhabdom turns the proximal photoreceptors, R5-8, which contain the PrL, into R or dR receptors. This can be well understood from the waveguide properties of the rhabdom (31). The light-absorbing pigment surrounding the rhabdom will act as a spectral filter by absorbing the boundary wave. The effect of the spectral filter is much stronger in the proximal tier than in the distal tier, simply because of the path length.

The short-wavelength receptors are organized in a very different way. *In situ* hybridization of mRNAs encoding PrUV, PrV and PrB (*Pieris rapae* *UV*, *Violet* and *Blue*), carried out in three consecutive sections (Fig. 6a–c), revealed that probes for the short-wavelength visual pigments unambiguously labeled the photoreceptors R1 or R2. The labeling pattern with the probe for the PrUV mRNA revealed three ommatidial types (I, II and III in Fig. 6a). The R1 and R2 in type I ommatidia were labeled by the PrB and PrUV probe (Fig. 6a,c), and their spectral sensitivities peak in the ultraviolet and blue wavelength range, respectively (Fig. 6d,f). The sensitivity spectra of the UV and B receptors nicely match the absorption spectra expected for visual pigments peaking at 360 nm (R360) and 453 nm (R453), and accordingly PrUV and PrB are equivalent to R360 and R453, respectively (Fig. 6d,f). The R1 and R2 in type II ommatidia were both labeled by the PrV probe (Fig. 6b), but only in the female the spectral sensitivity matches the absorption spectrum expected

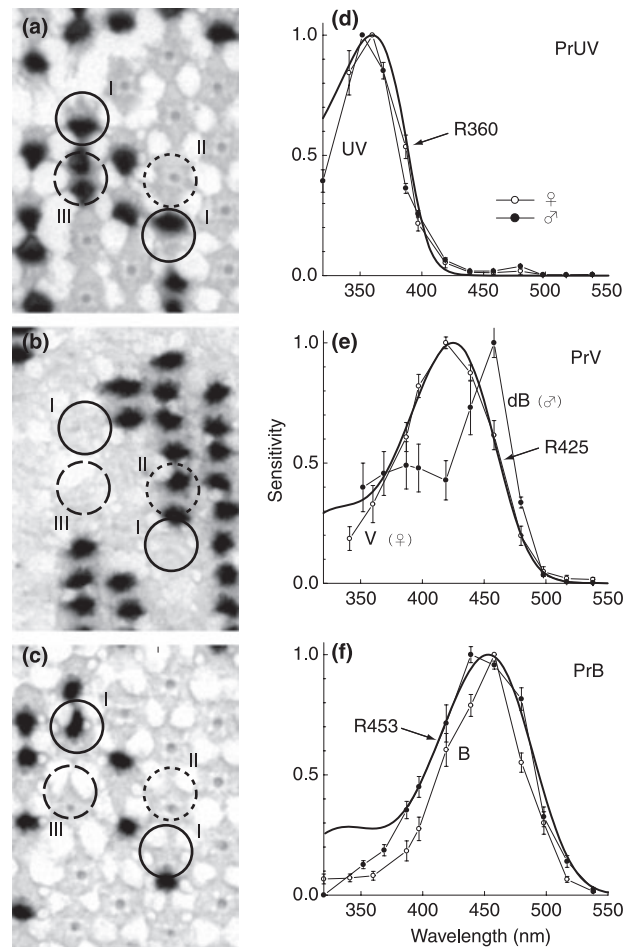


Figure 6. Short-wavelength photoreceptors of *Pieris*. *In situ* hybridization of the PrUV (a), PrV (b), and PrB (c) mRNAs in three consecutive sections. Circles indicate identical ommatidia. Three ommatidial types are evident in (a) (solid circle = type I, dotted circle = type II, broken circle = type III). Same in b and c. (d) Spectral sensitivity of the UV receptor (filled circles = males, open circles = females, same in e and f) and the absorption spectrum (thick line, same as e and f) of PrUV. (e) Spectral sensitivities of photoreceptors containing PrV, which are V receptors in females and dB receptors in males. (f) B receptor spectral sensitivity and PrB absorption spectrum.

for a visual pigment, namely that of a violet rhodopsin, R425 (Fig. 6e). The spectral sensitivity of the R1 and R2 of males is double-peaked blue, and thus these receptors are called dB receptors (Fig. 6e). As mentioned above, the type II ommatidia of males fluoresce under violet (420 nm) epi-illumination (Fig. 4c). Optical modeling provided unequivocal evidence that the fluorescing pigment acts as a violet-absorbing spectral filter, which modifies the PrV-containing receptors in type II ommatidia of males into dB receptors (24). Both R1 and R2 of type III ommatidia are labeled by the PrB probe (Fig. 6c), and their spectral sensitivities match the absorption spectrum of visual pigment R453.

PAPILIO: PHOTORECEPTOR DIVERSIFICATION VIA MULTIPLE VISUAL PIGMENTS

The compound eye of the Japanese swallowtail butterfly *P. xuthus* bears three types of ommatidia, as in *Pieris rapae*

and other insects, which is recognizable from the characteristic pigmentation (Fig. 7a). The first ommatidial type has red pigment around the rhabdom (type I). In the second type red pigment also surrounds the rhabdom, but there is in addition an ultraviolet-fluorescing pigment in the distal portion of the rhabdom (type II). The third type has yellow perirhabdomal pigment (type III). The red and yellow pigments function as spectral filters, and therefore the ommatidia look brightly colored when illuminated with white light from the proximal side (Fig. 7b). The red, less-saturated red, and yellow facets in Fig. 7b correspond to type I, II and III ommatidia, respectively. When observed under UV epi-illumination, the type II ommatidia emit a strong, whitish fluorescence, like glowing stars in the night sky (Fig. 7c). The fluorescing substance is most likely 3-hydroxyretinol, which is somehow deposited in the distal portion of the rhabdoms of the type II ommatidia (32).

The ommatidial heterogeneity is also clear in histological *in situ* hybridization studies of mRNAs of visual pigment opsins. Fig. 7d,e are two consecutive sections through the proximal tier of the retina, each labeled with the probe for PxL2 (*P. xuthus* long wavelength opsin 2, which is an opsin for the green absorbing visual pigment) mRNA, and with the probe for PxL3 (opsin for the red absorbing visual pigment) mRNA. Both probes labeled the proximal R5-8 photoreceptors. The PxL2 probe labeled the R5-8 densely in type III ommatidia, but only lightly in type II ommatidia (Fig. 7d). The PxL3 probe labeled R5-8 in both type I and II ommatidia (Fig. 7e). Double labeling of R5-8 in type II ommatidia with the probes for PxL2 and PxL3 mRNA is not the result of cross-reactivity of the probes, because some photoreceptors were solely labeled by either one of the probes (Fig. 7d,e).

The finding that the R5-8 of type II ommatidia express PxL2 as well as PxL3 mRNAs is at variance with the generally accepted concept in vision research that single photoreceptors contain a single type of visual pigment. However, the type II R5-8 photoreceptors clearly violate the dogma. Actually, the *Papilio* eye has multiple photoreceptor classes expressing more than one visual pigment. The R3, R4 and R9 of all ommatidial types co-express two G-opsins, PxL1 and PxL2, as summarized in Fig. 7 (33).

Among the six spectral receptors so far identified in the *Papilio* eye (Fig. 8), the broad-band (BB) receptors and the V receptors are extraordinary, for the spectral sensitivity of each of these receptors cannot be simply explained by a visual pigment absorption spectrum. The BB spectrum has a

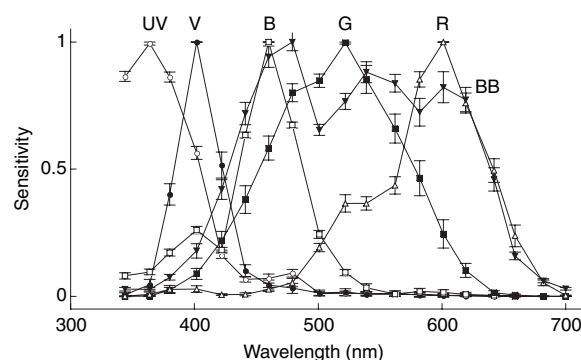


Figure 8. Six classes of spectral receptors identified in the *Papilio* retina.

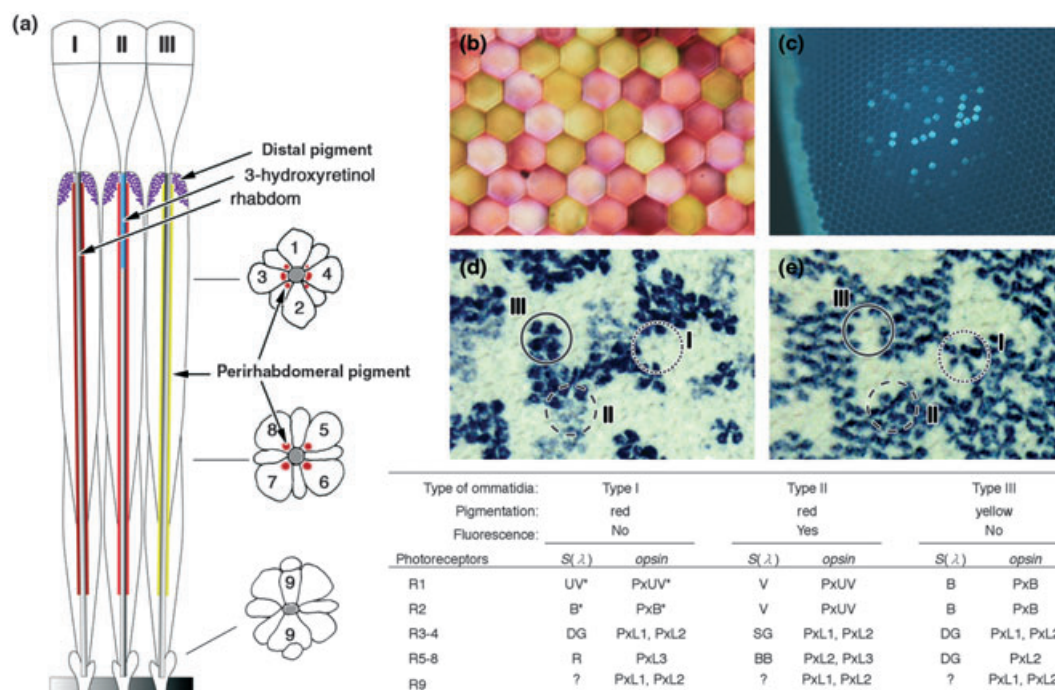


Figure 7. Anatomy of the *Papilio* eye. (a) Diagram of three types of ommatidia (I, II, III). The ommatidia are tiered with four distal photoreceptors (1–4), four proximal photoreceptors (5–8) and one basal (9) photoreceptor. The R1 and R2 contain distally purple pigment granules (distal pigment). (b) An eye slice back-illuminated with white light. (c) UV-induced fluorescence, emitted by 3-hydroxyretinol, marks the type II ommatidia. (d,e) *In situ* hybridization of PxL2 (d) and PxL3 (e) mRNA in adjacent sections through the proximal tier. R5-8 of type II contain both mRNAs. Table: Spectral sensitivities and visual pigment of photoreceptors R1-9 in the three types of the ommatidia. UV, ultraviolet; V, violet; B, blue; SG, single-peaked green; DG, double-peaked green; R, red; BB, broad-band. For visual pigments, see Fig. 2.

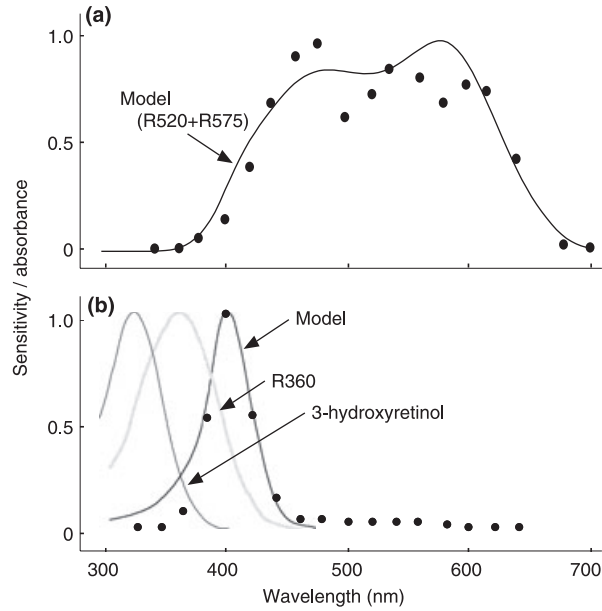


Figure 9. Spectral origin of the BB receptor (a) and the V receptor (b) in *Papilio*.

half-bandwidth of about 200 nm, which is much too broad for a visual pigment. The spectral sensitivity of the V receptors, peaking at 400 nm, is abnormally narrow compared to the absorption spectrum of an R400 visual pigment.

What is the spectral origin of these unique receptor classes? By combining electrophysiology and histology, we localized the BB receptors in the R5-8 photoreceptors of type II ommatidia: they are the cells double labeled with the PxL2 probe and the PxL3 probe. The BB sensitivity can be well explained by assuming that both the G and R visual pigments function simultaneously in the phototransduction process (Fig. 9a) (34). The V receptors are exclusively found in type II ommatidia (35). *In situ* hybridization revealed that the UV receptors and V receptors share a UV visual pigment, PxUV (36), and we therefore assumed that the V receptors indeed contain a UV-absorbing visual pigment. As noted above, the UV-absorbing 3-hydroxyretinol is concentrated distally in the

rhabdom of type II ommatidia. By assuming that the 3-hydroxyretinol acts as a UV-filter for the V receptors, we could reasonably well reconstruct the 400 nm-peaking, narrow spectral sensitivity curve (Fig. 9b). The presence of a UV filter also explains the extremely reduced sensitivity in the UV region of the BB receptor spectral sensitivity (Fig. 9a).

TWO STRATEGIES FOR SEEING A COLORFUL WORLD

The spectral organization of compound eyes appears to be specific for the insect species studied so far into great detail. Fig. 10 summarizes the relationship between visual pigment absorption spectra and photoreceptor spectral sensitivities. The cases of *Apis mellifera*, *Vanessa cardui* and *Manduca sexta* are simple, with three visual pigments that are directly correlated with three spectral receptor classes. This is probably also the case in *Bombus impatiens* (21). As these species have no receptors sensitive in the red wavelength region, the extent of their visible wavelength range is rather limited. Assuming that the simple trichromatic receptor set is the prototype in terms of the spectral organization of the compound eye, *Pieris* and *Papilio* have expanded their limited visible range by adding red receptors at the long wavelength side. Indeed, *Papilio* takes advantage of its red sensitivity by utilizing red flowers as a valuable nectar source (37), something honeybees are unable to do. *Pieris* possesses even two different red receptor classes, probably for enhanced discrimination of similar reddish colors. Insertion of an extra class of spectral receptors in the short-wavelength range between two extant spectral classes also occurs: this will obviously enhance the wavelength discriminability in the violet-blue.

The present research has made clear that there are at least two distinct strategies to acquire additional spectral receptor classes to the prototype set of UV, B and G receptors. Firstly, the multiple opsin strategy, where opsins of different amino acid sequences are produced, probably by gene multiplication (38), and secondly the filter strategy, that is the introduction of photostable spectral filters meanwhile keeping the visual pigment opsins unchanged. *Papilio* butterflies apparently have taken the multiple opsin strategy to add red receptors as the first choice, for they have an R receptor specific opsin (PxL3 in

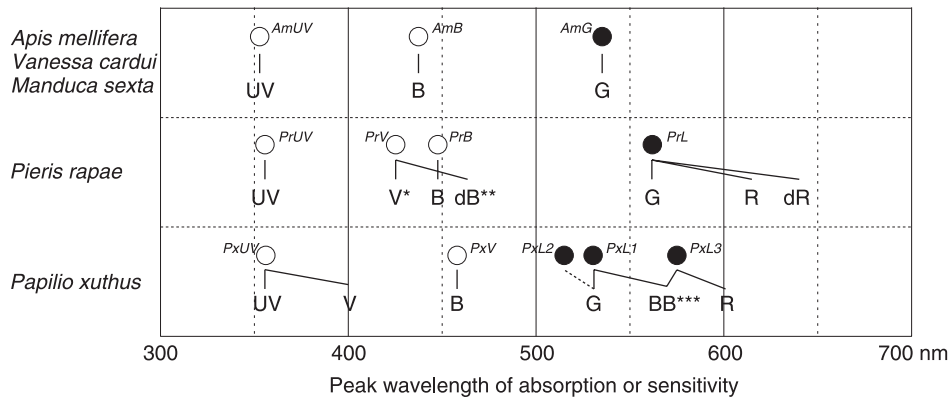


Figure 10. Relationship of visual pigments and spectral receptors. Opsin genes are indicated in italics, and photoreceptor spectral classes are in bold capitals. *Apis* and *Vanessa* are rather simple, but *Pieris* produces variable receptors by extensive filtering, whereas *Papilio* produces additional receptors, both by filtering (V) and by opsin duplication (R). The V receptor of *Pieris* (*) only exists in females, and its male version is the dB receptor (**). See Fig. 6. The characteristic coexpression of multiple opsins is only seen in *Papilio* (***).

P. xuthus, PglRh3 in *P. glaucus*) in addition to two G opsins (PxL1 and PxL2 in *P. xuthus*, PglRh1 and PglRh 2 in *P. glaucus*) (26). *Papilio* also uses the filter strategy to some extent. The R receptor, with peak sensitivity at 600 nm, appeared to contain an R575 visual pigment. The 25 nm shift of the peak is because of the filtering effect of the red perirhabdomeral pigment (39). *Pieris rapae*, on the other hand, relies solely on the filter strategy for producing R and dR receptors. Although the filter strategy should reduce the sensitivity of photoreceptors, many butterfly species seem to have adopted this approach. Examples are the monarch *Danaus plexippus* (10), the postman butterfly *Heliconius melpomene* (Heliconidae), the bush brown *Bicyclus anynana* (Satyridae) (40), and the pale grass blue *Zizeeria maha* (Lycaenidae) (5), where reddish pigmentation is evident in R5-8 proximal photoreceptors in a subset of ommatidia.

The situation is opposite for short-wavelength receptors. As in *Apis* and *Vanessa*, *Papilio* has two short wavelength-absorbing opsins, PxUV and PxV, but it has three classes of spectral receptors: UV, V, and B. A UV absorbing filter has the key role to produce the V sensitivity from the PxUV-containing photoreceptors. *Pieris rapae* also possesses V receptors, but here a specific visual pigment, PrV, has been developed. Although *Pieris* basically takes the multiple opsin strategy, the filter strategy is used to produce the characteristic sexual dimorphism in short-wavelength receptors.

CONCLUDING REMARKS

In this study, we compared the spectral organization of the compound eyes of several flower-visiting species. The organization seems quite variable, and it is therefore rather difficult to extract general principles at the present stage. We clearly have to collect equivalent and detailed data sets from other insect species, including nonflower visitors.

In the research field of molecular evolution of color vision, the amino acid sequences of opsins are always at the center of the discussion. The opsin primary structure undoubtedly provides the principal information about the spectral sensitivity of the photoreceptor, but the filter mechanisms in the *Pieris* eye indicate that photostable pigments can crucially affect the spectral sensitivity by acting as a powerful filter (23). Other examples where pigments acting as spectral filters have been convincingly demonstrated are the cone photoreceptors of birds and reptiles (41–44), as well as the rhabdoms of mantis shrimp (45,46). However, conclusive statements on the role of filtering pigments in “the evolution of color vision” must await careful behavioral proofs of the presence of real color vision, that is, the ability to discriminate visual stimuli based on the chromatic content regardless of the brightness.

After submitting this review, the opsin distribution in the compound eye of the lycaenid species *Lycaena rubidus* was reported (47). The authors identified three opsins for short wavelength-absorbing visual pigments and one opsin for long wavelength visual pigment, as in *Pieris rapae* (23,24). However, their distribution is different from *Pieris*: the three short wavelength-absorbing types conform to six distinct ommatidial types, whereas *Pieris* has only three. It was also shown that some photoreceptors of *Lycaena* co-express a short-wavelength type and the long-wavelength type. Detailed electrophysiological recordings of receptor spectral sensitivities as well as

behavioral experiments will be necessary to show whether these unique photoreceptors are involved in color vision.

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